In claims 31, 32 and 33, line 1, after "The" and before "cell" please insert -- isolated --.

37. (Once amended) The isolated cell of claim 36, wherein said coding region that is operably linked to said transcriptional regulatory sequence is selected from the group consisting of E1a, E1b, E2, and E4 coding regions.

In claims 38 and 39, line 1, after "The" and before "cell" please insert -- isolated --.

40. (Once amended) A method of producing a tissue-specific replication-conditional adenovirus virion [capable of tissue-specific replication], said virion comprising a heterologous tissue-specific transcriptional regulatory sequence operably linked to the coding region of a gene that is essential for replication of said virion, comprising culturing the isolated cell of claim ~~30~~ and recovering said virion from ~~said cell~~ the culture.

Remarks

I. Support for Amendments

The foregoing amendments to the specification have been made to correct errors in the names of the plasmids shown in Figure 1 where they are referenced at page 33 of the specification; to correct an obvious typographical error in the name of an inventor of, and to insert the U.S. application number for, commonly owned, co-pending U.S. Application No. 08/458,403 which is incorporated by reference at page 39; and to indicate the SEQ ID NOs next to the DNA sequences disclosed at page 41 of the specification, in compliance with 37 C.F.R. §§ 1.821

and 1.825. Support for the amendments at page 33 may be found in the plasmid designations shown in Figure 1 as originally filed, and support for the amendments at page 39 may be found at page 33, lines 14-16, of the specification as originally filed. Hence, these amendments to the specification do not add new matter.

Support for the foregoing amendments to the claims may be found throughout the specification. Specifically, support for new claims 41 and 42 may be found, *inter alia*, at page 22, lines 8-29, at page 25, lines 18-27, and at page 26, lines 1-11; support for new claims 43 and 44 may be found at page 8, line 10, to page 9, line 4, and at pages 28-30; support for the amendments to claims 1, 19, 29, 30 and 40 may be found, *inter alia*, at page 6, line 14, to page 9, line 4, and at page 9, lines 11-12; and support for the amendments entering the language "isolated cell" into claims 19-22, 26-28, 30-33 and 37-39 may be found at pages 28-30, and throughout Example 1 at pages 29-36. The amendments to claims 10 and 11 have been made solely to correct obvious errors in antecedent basis in both claims, which depend from claim 9. Accordingly, the present amendments do not add new matter and their entry is respectfully requested.

II. Status of the Claims

New claims 41-44 are sought to be added, claims 4-6, 13-15, 23-25, and 34-36 are canceled, and claims 1, 7, 9, 10, 11, 16, 19-22, 26-33, and 37-40 have been amended by the foregoing amendments, which do not introduce new matter. Claims 1-3, 7-12, 16-22, 26-33 and 37-44 are thus pending in the present application, with claims 1, 9, 19, 30, 41 and 42 being the independent claims.

III. The Claimed Invention

The invention as claimed relates generally to recombinant vectors, particularly recombinant adenovirus vectors. The invention as claimed relates more specifically to replication-conditional adenovirus vectors, particularly such vectors that undergo tissue-specific replication. The vectors of the invention preferably comprise a heterologous tissue-specific transcriptional regulatory sequence operably linked to the coding region of a gene that is essential for replication of the vectors. The invention as claimed also relates to isolated cells, particularly cell lines, containing such replication-conditional adenovirus vectors. The invention as claimed also relates to methods for distributing a polynucleotide *in vivo* comprising introducing such vectors into the cells or tissues of an animal. In one preferred embodiment, such methods may be used to screen a tissue for the presence or absence of transcriptional regulatory functions that permit vector replication by means of the transcriptional regulatory sequence. In another preferred embodiment, such methods may be used to provide a therapeutic benefit from the presence of the vector *per se* or from heterologous gene products expressed from the vector and distributed throughout cells tissues into which the vectors have been introduced. The invention as claimed also relates to methods for killing a tumor cell *in vivo* or *ex vivo*, comprising introducing the adenovirus vectors of the invention into the tumor cell. The present invention thus provides compositions and methods, not previously available in the art, that may be used for a variety of clinical and diagnostic purposes.

IV. Summary of the Office Action

In the Office Action dated April 13, 1998, the Examiner has made two objections to the specification, and six rejections of the claims. Applicants respectfully offer the following remarks

to overcome or traverse each of these elements of the Office Action, in light of the above amendments.

V. Summary of the Interview

Applicants wish to thank Examiners Nguyen and Low for the time they took to discuss the present application and the outstanding Office Action during an interview conducted on August 12, 1998. During this interview, Applicants exhibited data demonstrating the invention as presently claimed. In addition, the merits of the Office Action were discussed, and the Examiners suggested amendments to the claims that may overcome the outstanding rejections.

VI. The Objection to the Specification Under the Sequence Rules is Accommodated

In the Office Action at page 2, the Examiner has objected to the specification because the specification does not conform to the sequence listing requirements of 37 C.F.R. § 1.821 since it discloses DNA sequences for which there is no indicated SEQ ID NO. As an example, the Examiner points out that the nucleotide sequences listed on page 41 of the specification are not referenced by SEQ ID NOs. By the foregoing amendments, the specification has been amended to recite appropriate SEQ ID NOs in Table 1 at page 41. As noted above, these amendments do not add new matter to the application. Hence, the Examiner's objection has been fully accommodated, and the specification is now believed to be in full compliance with the sequence listing rules under 37 C.F.R. §§ 1.821 and 1.825. Reconsideration and withdrawal of the objection are therefore respectfully requested.

VII. The Rejection of Claims 19-39 under 35 U.S.C. § 101 is Traversed

In the Office Action at pages 2-4, the Examiner has rejected claims 19-39 under 35 U.S.C. § 101 as being directed to non-statutory subject matter. Applicants respectfully traverse this rejection, in view of the foregoing amendments and the following remarks.

In making this rejection, the Examiner contends that the claims,

> absent the qualifying language of "isolated" or cultured *in vitro* include in [their] scope a human containing the cells transformed with the virus. A claim including within its scope a human being is not considered patentable subject matter . . . .

Office Action, paragraph bridging pages 2 and 3. By the foregoing amendments, claims 23-25 and 34-36 have been canceled, rendering moot this rejection as it applies to those claims. Applicants respectfully traverse this rejection as it applies to the remaining claims.

Independent claims 19 and 30, and thus the remaining claims that depend therefrom, are drawn to cells that have been transfected with a replication-conditional tissue-specific vector of the invention. That is, the claims are drawn to genetically engineered *cells*, not to whole organisms (including humans) containing such cells. One of ordinary skill would fully appreciate that these claims do not encompass humans containing such genetically engineered cells, based on the plain language of the claims and on the present specification. Since these claims do not encompass human beings, no Constitutional issues are raised or implicated by the claims, and the claims are in full compliance with the requirements of 35 U.S.C. § 101.

However, solely to expedite prosecution of the present application, claims 19 and 30, and the remaining claims that depend therefrom, have now been amended to recite an "isolated" cell as suggested by the Examiner. As noted above, these amendments do not add new matter. Hence, Applicants respectfully assert that these claims as amended are directed to statutory

subject matter. Reconsideration and withdrawal of the rejection of under 35 U.S.C. § 101 are therefore respectfully requested.

VIII. The Objection to the Specification for Improper Incorporation By Reference Is Traversed

In the Office Action at pages 3-4, the Examiner has objected to the specification under 35 U.S.C. § 112, first paragraph, for allegedly improperly incorporating subject matter by reference to a co-pending U.S. application. Applicants respectfully traverse this objection.

In making this objection, the Examiner contends that:

> [t]he specification is objected [to] because an attempt to incorporate subject matter into this application by reference to a US co-pending application without an identified serial number is improper because only allowed U.S. applications and U.S. Patents can be incorporated by reference . . . . However, given that the manufacture of plasmids PAVS21.TK1 and SE280-E1, which are fundamental to the construction of the tissue-specific adenovirus vectors (which is one of the claimed species recited in the claims) is described in Example 5 (p. 39) only by the improper incorporation of references to other, pending, patent applications which are not identified by any serial number, one skilled in the art requires an undue experimentation to obtain the plasmid DNA in order to practice the claimed invention.

Office Action at page 3, lines 10-13, and page 3, line 17, to page 4, line 2. Applicants respectfully disagree with these contentions, based on the foregoing amendments and the following remarks.

By the foregoing amendments, the specification has been amended at page 39 to insert the U.S. application number for the commonly owned, co-pending application of Kadan et al. (U.S. Application No. 08/458,403; hereinafter "the Kadan application"). The U.S. application number for the Kadan application is included in the present specification at page 33, lines 14-15, where the Kadan application is explicitly incorporated by reference. The Kadan application incorporated by reference at page 33 is the same as that referenced in Example 5 at page 39, as evidenced by

the listing of the same inventors, title and filing date on pages 33 and 39. An amendment made to specify the application number of a previously filed application which is referred to by, or incorporated by reference into, a co-pending application does not add new matter. *See In re Fouche*, 439 F.2d 1237, 1240 (CCPA 1971). Hence, the insertion of the application number for the Kadan application at page 39 of the present specification does not add new matter.

In addition, the Examiner's above-noted contention that "only allowed U.S. applications and U.S. Patents can be incorporated by reference" is incorrect. According to the Manual of Patent Examining Procedure (MPEP), "[a]n application for a patent when filed may incorporate 'essential material' by reference to (1) a U.S. patent or (2) a pending U.S. application . . . ." MPEP Rev. 3 § 608.01(p) (July 1997). Contrary to the Examiner's contention, there is no requirement that a pending application be *allowed* in order for the proper incorporation of that application into another, subsequently filed application. Instead, under MPEP § 608.01(p) all that is required for proper incorporation by reference to a U.S. application is (1) that the referenced application be *pending*, and (2) that "an identification of the referenced patent, application, or publication" be included in the referencing application. *Id.* Since the Kadan application was pending at the time of filing of the present application, since the title and filing date for the Kadan application are provided at pages 33 and 39 of the present specification, and since the application number for the Kadan application is provided at page 33 of the specification as filed and at page 39 of the specification as now amended, the Kadan application has been properly identified in the present application. Thus, under MPEP § 608.01(p), the incorporation of the methods for producing plasmids PAVS21.TK1 and SE280-E1, by reference to the Kadan application, is proper in the present application.

In view of the foregoing remarks, Applicants respectfully assert that the incorporation of subject matter into the present application by reference to a co-pending U.S. Application was made in full compliance with 35 U.S.C. § 112, first paragraph. Applicants therefore respectfully request that the objection to the specification for improper incorporation by reference be reconsidered and withdrawn.

IX. The Rejections Under 35 U.S.C. § 112, First Paragraph, Are Traversed

In the Office Action at pages 4-9, the Examiner has rejected claims 1-40 under 35 U.S.C. § 112, first paragraph, for lack of sufficient written description. Applicants respectfully traverse these rejections, in view of the foregoing amendments and the following remarks.

A. The Rejection of Claims 1-40

In the Office Action at page 4, the Examiner first contends that:

> [t]he application provides guidance as to the construction of the claimed vectors, but does not demonstrate with evidence that any of the disclosed vectors comprising a tissue specific regulatory sequence(s) is specifically activated for replication in a target tissue.

Applicants respectfully disagree with this contention. Example 1 of the present specification (see pages 32-35) demonstrates the construction of an adenovirus vector comprising the hepatoma-specific α-fetoprotein (AFP) promoter operatively linked to the adenoviral E1a gene, to produce the AVAFPE1a (also known as AvE1a04i) construct. In this same Example, at pages 35-36 and in Figure 3, the specification demonstrates that this vector replicates and produces cytopathic effects specifically in HuH7 cells which express AFP, but not in A549 cells which do not express AFP. Hence, the AVAFPE1a (AvE1a04i) construct is a vector comprising a tissue-specific

regulatory sequence (the AFP promoter) that is specifically activated for replication only in AFP-expressing target tissues (such as HuH7 hepatoma cells).

These results were confirmed in a broader survey of AFP-expressing (*i.e.*, "AFP$^+$") and AFP-nonexpressing (*i.e.*, "AFP$^-$") cell lines, as depicted in Figures A-H accompanying the Declaration Under 37 C.F.R. § 1.132 of Paul L. Hallenbeck that is attached hereto. Initially, variety of cell lines and primary human cells were examined for their ability to express AFP. As shown in Figure A, the human hepatoma cell lines Hep3B, HepG2 and HuH7 expressed significant quantities of AFP, while non-hepatoma cell lines and primary human lung cells did not. When these cell lines and primary cells were transfected with the positive control, replication-competent adenovirus construct Add1327 (which resembles wildtype adenovirus in its ability to replicate constitutively), all of the cell lines tested were capable of supporting the replication of Add1327 and demonstrated cytopathic effects (Figure B). When these same cells and cell lines were transfected with the negative control, replication-defective adenovirus construct Av1nBg01v (which is deficient in E1a production; this same vector is also referred to as AV1LacZ4), the virus only replicated in AE1-2a cells (also known as A30 cells), which complement the E1a deficiency of the virus in *trans* (Figure C). These cells and cell lines were then transfected with the tissue-specific, replication-conditional adenoviral vector construct AvE1a04i (the same vector referred to above as AVAFPE1a), containing the E1a gene operably linked to the AFP promoter. As shown in Figure D, this vector construct was able to replicate and induce a cytopathic effect in the AFP$^+$ cell lines Hep3B and HepG2, and in the positive control AE1-2a (A30) cell line, but not in any of the AFP$^-$ cell lines in which the E1a gene was not activated. Under these conditions, the AFP$^+$ cell line HuH7 did not demonstrate substantial viral replication and CPE; however, when HuH7 cells were transfected and cultured for longer periods of time, or were transfected with a

higher viral MOI, the AvE1a04i construct was able to replicate and induce a cytopathic effect in these cells as well (data not shown). Hence, production of high titer adenovirus stocks comprising the AvE1a04i vector has been accomplished in Hep3B, HepG2, and HuH7 cells, all of which are AFP^+. In contrast, the AFP^- A30 cell line did not demonstrate viral replication or CPE, even when cultured for longer periods of time or when infected with a higher viral MOI. These results are confirmed in the photomicrographs depicted in Figure E: the Add1327 construct induced cytopathic effects in all cell lines tested, regardless of their expression levels of AFP; the AVE1a04i construct replicated specifically in AFP^+ cells (Hep3B) and in positive control cells expressing E1a in *trans* (A30), but not in AFP^- cells (Chang); and the replication-defective adenovirus Av1nBg01v (referred to in Figure E as AV1LacZ4) was only capable of replication in positive control cells expressing E1a in *trans* (A30).

Results have also been obtained that demonstrate the success of the claimed vectors and methods in inducing viral replication and cytopathic effects in primary cells and in tumor cells *in vivo*. In a first set of studies, adult swine and human hepatocytes (which do not express AFP), and the fetal swine hepatocyte cell line PICM19 (which constitutively express AFP), were transfected with the positive control Add1327 virus. As shown in Figure F, the virus replicated and induced cytopathic effects in all of the cells tested, regardless of their level of AFP expression. When these cells were transfected with the tissue-specific, replication-conditional adenoviral vector construct AvE1a04i, replication and cytopathic effects were only observed in the AFP^+ PICM19 cell line, and not in those cells that were AFP^-. Similar results were obtained when AFP^+ tumor cells (Hep3B) or AFP^- cell lines (Chang) were pre-transfected *in vitro* with the AvE1a04i construct and then injected into nude mice. As shown in Figure G, tumors developed only in those mice injected with pre-treated Chang cells (in which vector replication and cell killing do

not take place), but not in mice injected with pre-treated Hep3B cells (which express AFP and therefore in which the AvE1a04i vector is able to replicate and kill the cell). Finally, these results were extendable to treatment of pre-established tumors *in vivo*. As shown in Figure H, when Hep3B cells were injected into nude mice and the mice either not treated or only mock (vehicle) transfected, all of the mice died with 56 days. Injection of the AvE1a04i vector into these same mice, however, provided significant enhancement of viability of these mice: after 98 days, over 50% of the mice remained alive, indicating that the AvE1a04i vector construct is able to localize to the tumor tissue in these mice, selectively replicate therein and ablate the tumors.

Taken together, these results demonstrate that by following the methods described in the present specification, one of ordinary skill in the art could make and use vector constructs comprising a tissue-specific regulatory sequence (such as the AFP promoter) that is tissue-specific for replication. Therefore, the above-noted evidence provided by the present specification and the accompanying Rule 132 Declaration indicates that the specification fully enables vectors that are specifically activated for replication in a target tissue, such as those that are presently claimed.

The Examiner also contends in this portion of the rejection that "development of effective transcriptionally targeted vectors remains unpredictable." Office Action at page 5, lines 1-2. In support of this contention, the Examiner cites Vile *et al.* (*Molec. Med. Today 4*:84-92 (1998) (Document U on the Form PTO-892 attached to Paper No. 6; hereinafter "Vile") and Russell *et al.* (*Eur. J. Cancer 30A*:1165-1171 (1994) (Doc. AT15, of record; hereinafter "Russell") as indicating the alleged unpredictability of such an approach. The Examiner then concludes that

> it is not apparent how one skilled in the art determines which of the tissue-specific replication-conditional vectors recited in claim 1 is tissue specific for replication without undue experimentation on the basis of applicant's disclosure, particularly given the doubts expressed in the art of record.

Office Action at page 5, lines 14-17. Applicants respectfully traverse the Examiner's contentions. As noted above and as demonstrated in Figures A-H contained in the accompanying Rule 132 Declaration, one of ordinary skill could readily produce vectors that conditionally replicate in a tissue-specific fashion *in vitro* and *in vivo* by following the methods described in the present specification, without resorting to undue experimentation. In addition, Applicants respectfully disagree with the characterizations of Vile and Russell as they allegedly relate to the presently claimed vectors and methods.

In citing Vile, the Examiner points to the statements at page 90, column 1 of this reference (*see* Office Action at page 5, lines 2-8) that certain challenges remain to the development of effective transcriptionally regulated target-specific vectors. However, when Vile is read in its entirety, one of ordinary skill would see this reference as *supporting*, rather than questioning, the feasibility of the presently claimed vectors and methods. For example, at page 89, third paragraph under the section entitled "Transcription," Vile states

> Many examples now exist where a tissue-specific promoter has been used to drive expression of therapeutic genes. We have used the promoter of the melanocyte-specific tyrosinase gene to direct expression of genes in melanoma cells following systemic and localized administration of plasmid or retroviral vectors.

(Footnotes omitted.) Analogously, immediately following the identification of certain technical challenges in the section at page 90 referred to by the Examiner, Vile notes that solutions to these challenges are readily available, including the approaches taken in the present invention:

> These restrictions might be eased with the emerging development of vectors that can take large amounts of inserted DNA, including artificial chromosomes and heavily deleted 'gutless' adenoviral vectors.
>
> An elaboration of this approach is the use of inducible promoters so that gene expression can also be temporally targeted . . . . Increasingly, more elaborate synthetic promoters are becoming available that can be activated/repressed by

> administration of exogenous agents *or the presence of cell-specific factors*, thereby offering the opportunity of both spatial and temporal targeting of gene expression.

Vile at page 90, col. 1, lines 33-38, 40-44 (emphasis added, footnotes omitted). Thus, far from questioning the predictability or operability of vectors such as those of the present invention, Vile actually provides a review of the literature where such vectors have been successfully produced and used. Hence, the Examiner's contentions that Vile suggests that the development of effective transcriptionally targeted vectors remains unpredictable represent a reading of Vile that is, at best, out of context and probably contrary to the teachings of Vile viewed as a whole.

Similarly, in citing Russell, the Examiner points to the statements in this reference at page 1168, column 2, that cell-specificity of promoters used in the vector constructs was partially lost after replication of the viral DNA. These statements are used by the Examiner to support the contention that one of ordinary skill would have to undertake undue experimentation to make and use the tissue-specific replication-conditional vectors of the present invention. As was the case for the excerpt from Vile, however, one of ordinary skill reading these statements within the context of the complete Russell reference would not likely reach the same conclusion.

Within the same paragraph on page 1168 of Russell that is cited by the Examiner, the authors state that despite studies demonstrating that challenges may exist to developing tissue-specific replication-conditional vectors,

> these studies strongly suggest that with careful attention to the stoichiometry and kinetics of gene regulation by cellular transcription factors, it should be possible to engineer the promoters of replicating vectors for tissue-specific, transformation-dependent expression.

Russell at page 1168, col. 2, last sentence in second paragraph under "Viral promoter engineering." Thus, even Russell acknowledges that the challenges pointed to by the Examiner

should not be so difficult to overcome that the production and use of tissue-specific, replication-conditional vectors such as those of the present invention would be remain unpredictable or require undue experimentation. As one of ordinary skill would appreciate, this acknowledgment by Russell has been borne out by the present invention.

Further in this portion of the Office Action, the Examiner also contends that :

> it is not apparent how one skilled in the art determines which of the tissue-specific replication-conditional vectors recited in claim 1 is tissue specific for replication without undue experimentation on the basis of applicant's disclosure, particularly given the doubts expressed in the art of record.

Office Action at page 5, lines 14-17. Applicants respectfully disagree with these contentions.

First, as noted above, when the art that is of record (*i.e.*, Vile and Russell) is read properly, this art does not express "doubts" regarding determination of the tissue-specificity of vector constructs such as those that are presently claimed. To the contrary, as also noted above, the art of record actually acknowledges that vectors capable of tissue-specific replication should be able to be produced by an ordinarily skilled artisan, for example by following methods such as those of the present specification without undue experimentation.

Second, the present specification teaches in detail how to determine whether or not a particular vector construct is tissue-specific for replication. For example, at page 22, lines 1-2, it is disclosed that when a vector construct is contained in a target tissue expressing the proper activating or derepressing factors, the virus may replicate and packaging of DNA into virions may also occur. At pages 27-28, the specification describes methods useful for determining whether or not the claimed vectors will replicate in a specific tissue. At pages 31-32, the specification teaches that well-known techniques may be used to determine whether a particular vector construct replicates in a particular target tissue. In Example 1, at pages 35-36, the specification

provides methods and data demonstrating successful discrimination between tissues in which the vector constructs replicate (*e.g.*, HuH7 cells) and those tissues in which the constructs do not replicate (*e.g.*, A549 cells). Finally, in Example 2 at pages 36-38, the specification teaches methods suitable for determining the ability of a vector construct to replicate in tissues (*e.g.*, MCF-7 cells) expressing the DF3 enhancer. Thus, contrary to the Examiner's contention, one of ordinary skill in the art could readily determine, using the teachings of the present specification and without resorting to undue experimentation, which of the claimed vectors are tissue-specific for replication.

Further in this portion of the Office Action, the Examiner also contends that:

> [w]ith regard to claims directed to tissue-specific replication-conditional adenoviral vectors (which is a preferred species of the claimed invention), the adenoviral genes, E1b, E2, E1A, and E4 (all essential for replication of adenovirus vectors) encode proteins whose functions are dissimilar with each other. Each of these genes or regions require a certain level of expression to support adenoviral replication. Note also the application indicates at page 6 that expression levels of adenoviral genes essential for replication, *e.g.*, E1 and E4, must be carefully regulated in order to averse toxicity effect of the adenoviral genes upon the cells or tissues transformed with adenovirus vectors. Since it is known in the art that tissue-specific promoters activate constitutive expression of a transgene in a target tissue, it is not apparent how the expression levels of E4 gene, for example, are regulated from the claimed adenoviral vectors in order to avoid the toxicity to transformed cells (due to uncontrollable expression of E4 genes) prior to the replication and growth of the replication-conditional adenovirus vectors. There is no discussion in the specification of expression levels necessary to achieve appropriate expression for specific replication in a target tissue or cells *in vitro* and/or *in vivo*.

Office Action at page 5, line 17, to page 6, line 11. Applicants respectfully disagree with these contentions, for several reasons.

First, the present claims are drawn to vectors and methods that act to distribute a polynucleotide *in vitro* or *in vivo*, rather than being specifically drawn to methods of gene therapy.

As such, the claimed vectors and methods do not require a particular level of expression of the gene essential for vector replication (*e.g.*, E1 or E4) beyond that required for replication of the vector itself. Since the specification teaches in detail that the claimed vectors will replicate in a tissue-specific manner, one of ordinary skill in the art would understand and acknowledge that a level of expression of the gene essential for vector replication has, in fact, been achieved by the vectors and methods as claimed. Moreover, the Examiner has provided no evidence or sound scientific reasoning for doubting that the vectors produced by the methods described in the present specification will express a level of a replication-required gene (*e.g.*, E1 or E4) that is sufficient to promote vector replication. Absent such evidence or sound scientific reasoning that would provide one of ordinary skill with a reason to doubt the objective truth of the teachings of the present specification, the specification must be taken as being in compliance with the enablement requirement of 35 U.S.C. § 112, first paragraph. *See In re Marzocchi*, 439 F.2d 220, 223 (CCPA 1971). Hence, the present specification fully enables the claimed invention.

Second, the statements at page 6 of the present specification to which the Examiner points are intended only to indicate that if *both* E1 and E4 were expressed within the same cell, such as occurs in E1-complementing cell lines that are transfected with an E4-containing vector construct (or in E4-complementing cell lines transfected with E1 constructs), the cells will not survive. This statement does *not* indicate, however, that knowledge of the expression levels of E1 or E4 (or any other early viral gene, for that matter) is a prerequisite for the production and use of the claimed vectors. In fact, all that is required to determine the appropriate level of expression of E1, E2, E4 or any other early viral gene is an assay for measuring replication of the vector once it has been transfected into a given target cell. Such assays, in the form of measurements of DNA replication and measurements of cytopathic effects, are provided in detail in the present specification at pages

27, 31-32, and 35-36. Hence, one of ordinary skill could readily determine the amount of expression of any early gene, including adenoviral E1 or E4, that is required to produce the claimed tissue-specific, replication-conditional vectors.

Finally, the Examiner may have taken the above-noted statements at page 6 of the specification out of context. These statements in the specification only indicate that the expression levels of adenoviral genes essential for replication, *e.g.*, E1 and E4, must be carefully regulated in cells expressing *multiple* early genes, so that toxicity of the adenoviral genes in transformed cells can be avoided. These statements, as one of ordinary skill would appreciate, refers to the production of so-called "packaging" or "producer" cell lines that constitutively express multiple early genes. The production of such producer cell lines is, of course, inherently difficult due to the cytolysis that occurs upon viral replication. However, in order to produce early viral proteins (*e.g.*, E1 and E4) and replicate, the transfected cells need not necessarily survive for any longer than is necessary for the vector to replicate. In packaging cell lines, inducible promoters are routinely used to express early viral genes in transfected cells. There is no reason to believe that other early genes, besides E1, cannot be operatively linked to a tissue-specific promoter on an adenoviral vector construct and the construct then used to transfect cells for the production of progeny viruses or for the ultimate destruction of the transfected cell. Hence, regardless of which early viral gene is used in the claimed vector constructs, the outcome is the same -- a replication-conditional vector that undergoes tissue-specific replication.

In further support of the enablement of the claimed invention by the present specification, there have now been several reports demonstrating the use of the present methods to successfully produce tissue-specific replication-conditional vectors that selectively kill target cells *in vitro* and *in vivo*. For example, in Rodriguez *et al.*, *Cancer Res.* 57:2559-2563 (1997) (hereinafter

"Rodriguez," a copy of which is attached hereto as Appendix A), the investigators constructed vectors comprising enhancer/promoter regions from the human prostate-specific antigen (PSA, produced by prostatic carcinoma cells but not by normal prostate tissue), operatively linked to the adenovirus E1a gene. These vectors were transfected into a variety of cell lines, and were found to specifically replicate in, and kill, those tumor cell lines expressing PSA but not those that did not express PSA. In addition, injection of the vectors into nude mice bearing prostate tumors resulted in complete elimination of the tumors, while injection into mice bearing tumors that did not express PSA did not significantly inhibit tumor growth or survival. Thus, as concluded in Rodriguez, vectors constructed and used in methods according to the present invention demonstrate "tumor cell selectivity and tumor cell killing . . . . " Rodriguez at page 2561, col. 1, fourth full paragraph, lines 1-2.

Analogous results have been obtained in the following additional reports:

Reference	Viral Construct Used	Appendix:
Chen *et al.*, *J. Clin. Invest. 96*:2775 (1995)	DF3-HSV-*tk*	B
Wills *et al.*, *Canc. Gene Therap. 2*:191 (1995)	CMV-HSV-*tk*; AFP-HSV-*tk*	C
Siders *et al.*, *Cancer Res. 56*:5648 (1996)	tyrosinase-β-galactosidase	D
Kanai *et al.*, *Cancer Res. 57*:461 (1997)	AFP-cytosine deaminase	E
Lan *et al.*, *Cancer Res. 57*:4279 (1997)	CEA-cytosine deaminase	F

These reports demonstrate that vector constructs comprising a toxic gene (Chen, Wills, Kanai and Lan) or a marker gene (Siders) under control of a heterologous tissue-specific promoter will transfect, specifically replicate in, and kill or express the marker in, those tumor cell lines expressing the respective tissue-specific factor (*i.e.*, DF3 in Chen; AFP in Wills and Kanai;

tyrosinase in Siders; and CEA in Lan), but not those cells that did not express the tissue-specific factor.

The vectors used in the above-referenced reports are analogous to those of the present invention, wherein a gene essential for viral replication is operatively linked to a heterologous tissue-specific transcriptional regulatory sequence. Just as the vectors in the above-referenced report functioned to facilitate transcription of the toxic or marker gene in the transfected cells, under control of a heterologous promoter, the presently claimed vectors function to facilitate transcription of a gene essential for viral replication (and thus viral replication) under control of a heterologous transcriptional regulatory sequence such as a tissue-specific promoter. In addition, a variety of tissue-specific transcriptional regulatory sequences that may be used in the claimed vector constructs and methods are now known in the art (*see, e.g.*, Tables 1 and 2 at cols. 11-15 of U.S. Patent No. 5,728,379 to Martuza *et al.*, which was made of record by the Examiner in the present Office Action but which is post-filing art to the present application, as noted below). Since the above-referenced reports demonstrate expression of a non-cellular gene under control of a heterologous transcriptional regulatory sequence, and since a variety of tissue-specific transcriptional regulatory sequences are now known in the art, one of ordinary skill in the art would fully expect, and would have no reason to doubt, that the presently claimed vector constructs would work in the selective ablation of cells (particularly tumor cells) *in vitro*, *ex vivo*, and *in vivo*, by carrying out the claimed methods as fully described in the present specification.

Thus, it is clear from the results presented in the present specification, and from the results of the post-filing art cited above, that one of ordinary skill would be able, without undue experimentation, to make and use the claimed tissue-specific, replication-conditional vectors, and

the isolated cells containing and methods using such vectors. Reconsideration and withdrawal of this portion of the rejection are therefore respectfully requested.

B. The Rejection of Claims 3 and 11

In the Office Action at pages 6-7, the Examiner contends that:

> [w]ith regard to claims 3 and 11 drawn to the use of specific heterologous tissue specific promoters in the construction of the claimed vectors, an artisan, attempting to make and use the claimed vectors, would first look to the specification for guidance as to the availability of heterologous tissue-specific transcriptional regulatory sequences recited [in] claims 3 and 11. However, the specification is not enabling for the claimed vectors containing the promoters selected from [the] group consisting of tyrosinase, CEA, surfactant, and ErbB2 (recited in claims 3 and 11). The specification indicates that the promoters can be cloned and sequenced by PCR technology using the primers depicted in Table 1, however, it is not apparent how one skilled in the art clones, sequences, and employs the DNA sequence(s) encoding the functionally active promoters in the conditional replication vectors with a reasonable expectation of success and without undue experimentation, particularly since it is not apparent as to what is the length and the exact location of the DNA sequence(s) encoding the promoters, and as to whether there are more than one locus control regions and/or enhancers and/or silencers and/or promoter sequences involved in the make-up of the DNA sequences encoding the functionally active promoters recited in the claims. Note that Vile *et al.* (p. 90, column 1) teach that "the relevant locus control regions/enhancer/silencer/promoter sequences that control expression can be distributed over many kbp and within chromatin domains that are difficult to reproduce within the context of the vector systems".
>
> Thus, one skilled in the art cannot identify, without undue experimentation, a tissue in which all of the replication-conditional vectors recited in claim 1 are specifically replicated *in vitro* and/or *in vivo* by means of the transcriptional regulatory sequence contained in the vectors, particularly given the reasons set forth in the preceding paragraphs.

Applicants respectfully disagree with these contentions. As the Examiner acknowledges, the present specification teaches that a variety of tissue-specific promoters can be cloned from known

tissue or cell libraries via PCR using the primers depicted in Table 1. The "length and exact location of the DNA sequence(s) encoding the promoters" that the Examiner contends "is not apparent" from the present specification are well-known in the art. The following table is a representative, although not exhaustive, listing of references disclosing the sequences and locations of certain tissue-specific promoters that may be used in the present constructs; these references are either already of record, or are made of record in Applicants' First Supplemental Information Disclosure Statement (IDS) that is filed herewith:

Reference	**Tissue-Specific Promoter**	**IDS Doc. Ref. No.**
Nakabayashi *et al.*, *Mol. Cell. Biol. 11*:5885-5893 (1991).	α-fetoprotein (AFP)	AS22
Vile and Hart, *Cancer Res. 53*:962-967 (1993)	Tyrosinase	AS19
Vile and Hart, *Cancer Res. 53*:3860-3864 (1993)	Tyrosinase	AR19
Schrewe *et al.*, *Mol. Cell. Biol. 10*:2738-2748 (1990)	Carcinoembryonic antigen (CEA)	AS16
Richards *et al.*, *Human Gene Therapy 6*:881-893 (1995)	CEA	AR23
Abe *et al.*, *Proc. Natl. Acad. Sci. USA 90*:282-286 (1993)	DF3	AT20
Kovarik *et al.*, *J. Biol. Chem. 268*:9917-9926 (1993)	DF3	AS21
Manome *et al.*, *Cancer Res. 54*:5408-5413 (1994)	DF3	AR11
Harris *et al.*, *Gene Therapy 1*:170-175 (1994)	*erbB2*	AT7
Grooteclaes *et al.*, *Cancer Res. 54*:4193-4199	*erbB2*	AR21
Max-Audit *et al.*, *J. Biol. Chem. 268*:5431-5437 (1993)	Erythroid pyruvate kinase	AT21
Pang *et al.*, *Human Gene Therapy 6*:1417-1426 (1995)	Prostate-specific antigen (PSA)	AT22
Morishita *et al.*, *J. Biol. Chem. 270*:27948-27953 (1995)	*flt-1* VEGF receptor	AR22

In order to enable a claimed invention, a specification need not teach information that is well-known to those of ordinary skill in the art. *See Lindemann Maschinenfabrik v. American Hoist and Derrick*, 730 F.2d 1452, 1463 (Fed. Cir. 1984); *In re Wands*, 8 USPQ2d 1400, 1402 (Fed. Cir. 1988). One of ordinary skill in the art is deemed to know not only what is considered well-known, but also where to search for any needed starting materials. *See In re Howarth*, 210 USPQ 689, 692, (CCPA 1981). Since the sources, locations, and DNA sequences of a variety of tissue-specific regulatory sequences are known in the art (as noted above), and since the present specification teaches methods of obtaining such regulatory sequences using, *e.g.*, PCR with the primers demonstrated in Table 1, one of ordinary skill would be able to make and use the claimed vectors, cells and methods with a reasonable expectation of success and without undue experimentation. Hence, the present specification fully enables the invention as presently claimed, and reconsideration and withdrawal of this portion of the rejection are respectfully requested.

C. The Rejection of Claims 9-18

In the Office Action at page 7, lines 19-20, the Examiner contends that "the claims encompass gene-targeted therapy in any subject including a human and implanted cells containing the claimed vector for generating a therapeutic effect." The Examiner further contends that such gene-targeted therapy methods are unpredictable and that "without guidance from the specification the artisan would have been required [to] practice undue experimentation to construct and use the claimed vectors." Office Action at page 9, lines 14-16. The Examiner relies on the comments of Crystal (*Science 270*:404-410 (1995) (Doc. AS3, of record; hereinafter "Crystal")), Coghlan (*New Scientist 145*:14-15 (1995) (Doc. AT2, of record; hereinafter "Coghlan"), Günzberg *et al.* (*Molec. Med. Today 1*:410-417 (1995) (Doc. AS7, of record;

hereinafter "Günzberg"), Mastrangelo *et al.* (*Sem. Oncol. 23*:4-21 (1996) (Doc. AS11, of record; hereinafter "Mastrangelo"), and Ledley (*Hum. Gene Ther. 6*:1129-1144 (1995) (Doc. AS10, of record; hereinafter "Ledley"), to support the contention that targeted gene therapy remains unpredictable and to conclude that:

> [i]n view of the lack of guidance regard the administration parameters, lack of convincing data or working examples, breadth of the claims, state of the art and the unpredictability of the art, as set forth by the evidence presented above, undue experimentation would be required by one of ordinary skill to practice the invention as claimed.

Office Action at page 9, final paragraph. Applicants respectfully disagree with these contentions as they are applied to the invention as presently claimed.

The Examiner's contentions that "the claims encompass gene-targeted therapy," and that gene therapy allegedly remains unpredictable, are not relevant to enablement of the present claims. While Applicants teach that the presently claimed invention is operable *in vivo*, claims 9-18 do not specifically recite *in vivo* therapeutic efficacy or gene therapy *per se*, but instead recite the distribution of a polynucleotide in a tissue *in vivo*. Applicants wish to remind the Examiner that there are other uses for such a process which are *unrelated* to therapy, including but not limited to the production of a cell line that produces large amounts of the present vectors. Hence, the Examiner's contentions relating to the alleged unpredictability or inoperability of gene therapy methods are irrelevant to the present claims, which are not specifically drawn to methods of gene therapy.

Moreover, the nature of the present invention alone would not cause one skilled in the art to reasonably doubt its asserted usefulness in distributing polynucleotides to cells and tissues. The purpose of delivering nucleic acids to cells or tissues using the claimed methods, vectors and compositions does not suggest an inherently unbelievable undertaking or involve implausible

scientific principles. One skilled in the art would be without basis to reasonably doubt Applicants' asserted utility on its face, and Applicants should not be required to substantiate their presumptively correct disclosure to overcome the present rejection under 35 U.S.C. § 112, first paragraph. Additionally, according to the "Guidelines for Examination of Applications for Compliance with the Utility Requirement," issued by the Honorable Commissioner of Patents and Trademarks, "[i]f the applicant has asserted that the claimed invention is useful for any particular purpose and that assertion would be considered credible by a person of ordinary skill in the art, the Examiner should not impose a rejection based on § 101." *See Guidelines for Examination of Applications for Compliance with the Utility Requirement*, at Part I, Section B(2)(a), 1170 OG at 460-461 (January 24, 1995). Although the present Guidelines are specific to § 101 rejections, they are also to be applied to § 112, first paragraph, rejections. *See In re Brana*, 51 F.3d 1560, 1564 (Fed. Cir. 1995). Accordingly, under *Brana* and the Utility Guidelines, Applicants submit that claims 9-18 encompassing the potential use of, or methods involving, *in vitro* and *in vivo* applications of the claimed vectors are fully enabled by the specification as originally filed.

Moreover, even if the present claims *were* drawn to methods for targeted gene therapy, Applicants respectfully assert that the present specification fully enables one of ordinary skill to make and use such methods without resorting to undue experimentation. Undue experimentation is a conclusion that is reached by weighing many factual considerations. These considerations have been enumerated in *Ex parte Forman*, 230 USPQ 546, 547 (Bd. Pat. App. Int. 1986), and include (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims. Under *Forman*, a determination of

what constitutes undue experimentation in a given case requires the application of a standard of reasonableness having due regard for the nature of the invention and the state of the art. The test is not merely quantitative since a considerable amount of experimentation is permissible, if routine or if the specification in question provides sufficient guidance with respect to the direction in which the experimentation should proceed in order to enable one of ordinary skill in the art to practice the claimed invention. The emphasis is not on the term "experimentation" but on the term "undue." *See In re Wands*, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988).

Applicants respectfully submit that the present rejection of claims 9-18 has been based entirely on consideration of only one of the *Forman* factors: the predictability factor. Further, Applicants respectfully submit that insufficient evidence has been offered to support the conclusion that the invention is sufficiently unpredictable that it is not enabled to any degree. Accordingly, as discussed below, Applicants respectfully disagree with the rejection and submit that a *prima facie* case of nonenablement has not been established.

These above-noted comments by Crystal, Coghlan, Günzburg, Mastrangelo, and Ledley simply indicate that there was no *clinical* evidence, as of the date of filing of the present application, that genetic treatment has produced therapeutic benefits. There is simply no reason for the overextension of this opinion, as the Examiner has apparently done, to support the assertion that the present methods could not be used by the skilled artisan without undue experimentation. In making these contentions, the Examiner appears to suggest that for the claimed invention to be enabled, Applicants must demonstrate the clinical efficacy of the claimed methods (*i.e.*, that the methods are without obstacles, are safe, and are therapeutically effective) in order to overcome the outstanding enablement rejection. Applicants wish to remind the Examiner, however, that there is no requirement for clinical data to prove that an application is

in compliance with 35 U.S.C. § 112, first paragraph. In fact, description of *in vitro* and/or animal testing has been held to enable claims to *in vivo* therapeutic compositions and methods of their use. To this end, the Federal Circuit has stated that:

> In vitro testing, in general, is relatively less complex, less time consuming, and less expensive than in vivo testing. Moreover, in vitro results with respect to the particular pharmacological activity are generally predictive of in vivo test results, i.e., there is a reasonable correlation therebetween. Were this not so, the testing procedures of the pharmaceutical industry would not be as they are.

Cross v. Iizuka, 753 F.2d 1040, 1050 (Fed. Cir. 1985); *see also In re Brana*, 51 F.3d 1560, 1567-68 (Fed. Cir. 1995) (holding that animal testing results are sufficient to establish whether one skilled in the art would believe that a pharmaceutical compound has an asserted clinical utility for the purposes of compliance with the enablement requirement of 35 U.S.C. § 112, first paragraph). It is true that the inventions at issue in *Cross* and *Brana* did not involve vector constructs or methods for using such constructs. It is also true that the present Office Action does not *per se* state a requirement for clinical data. However, *Cross* and *Brana* are relevant to the present invention, despite the lack of a *per se* requirement for clinical data in the present Office Action, because in support of this rejection the Examiner has cited only the portions of the Crystal, Coghlan, Günzburg, Mastrangelo, and Ledley references regarding the lack of clinical evidence of success in using the claimed compositions and methods. To this end, *Cross* has been followed or cited with approval in a number of subsequent cases involving a variety of inventions; *see, e.g.*, *Bigham v. Godtfredsen*, 857 F.2d 1414, 1417 (Fed. Cir. 1988), *Fiers v. Revel*, 984 F.2d 1164, 1169 (Fed. Cir. 1993), *In re Ziegler*, 992 F.2d 1197, 1200-1201 (Fed. Cir. 1993), *In re Brana*, 51 F.3d 1560, 1564 n.11, 1565 (Fed. Cir. 1995), and *Fujikawa v. Wattanasin*, 93 F.3d 1559, 1563 (Fed. Cir. 1996). Similarly, *Brana* has been followed or cited with approval in a

number of cases, *e.g.*, *Ex parte Bhide*, 42 USPQ2d 1441, 1447 (Bd. Pat. App. Int. 1996), and *Sie v. Herskowitz*, 1998 U.S. App. LEXIS 3776 at *5 (Fed. Cir., March 5, 1998). Since neither the PTO nor the Federal Circuit have limited the essential holdings of *Cross* and *Brana* (as they relate to the enablement of therapeutic methods by *in vitro* data) to the specific facts or inventions in those cases, to do so in the present case would be unwarranted and contrary to the current state of the law.

The present specification clearly describes methods for preparation and use of the present vectors *in vitro, in vivo* and *ex vivo* (*see, e.g.*, Specification at pages 21-32). Under *Cross* and *Brana*, one of ordinary skill would thus recognize that the *in vitro* assays described in the present specification would be "generally predictive of *in vivo* test results," *Cross*, 753 F.2d at 1050, and thus would have a reasonable expectation that the claimed methods would be successful for the claimed *in vivo* therapeutic approaches.

Furthermore, at the time the invention was made, ample information was available to skilled artisans regarding various ways of "whole animal" treatment with transfection vectors carrying a gene of interest. It is not relevant to the inquiry at hand, *i.e.*, to the "patentability" of the claimed invention, that certain investigators (such as those relied upon by the Examiner above) were not satisfied with the level of efficacy and safety of gene therapy at the *clinical* level. It is an incorrect application of patent law for the Examiner to require Applicants to demonstrate routine, highly effective, and safe methods of gene therapy to support enablement of a claim to methods of using the claimed vectors to distribute a polynucleotide in a tissue *in vivo*. It is sufficient for patentability purposes that the invention can be practiced *at all* as taught in the specification and in light of the knowledge in the art. Whether the invention would meet agency

standards for routine medical application is an inquiry specifically reserved for the Food and Drug Administration, and is irrelevant to patentability. *See Brana*, 51 F.3d at 1567.

Applicants respectfully assert that the art is replete with various examples of successful transfection of nucleic acid molecules *in vivo*. Hence, it would be clear to the skilled artisan that the same types of transfections may now be done using the vectors, compositions and methods of the present invention to increase gene expression in cells and tissues *in vivo*, according to an embodiment of the claimed invention. Whether further research and development, however, would be required to perfect the available gene therapy methods is not material to the patentability of the claimed invention. It is, in fact, expected that such improvements to most inventions are required in order to develop a safe and desirable invention for routine use. In view of the foregoing remarks, Applicants therefore respectfully assert that claims 9-18 are fully enabled by the present specification, and that one of ordinary skill would not be required to undertake undue experimentation to make and use the invention as claimed.

D. Summary

In view of the foregoing amendments and remarks, Applicants respectfully assert that claims 1-40 are fully described and enabled by the specification as originally filed. Reconsideration and withdrawal of the rejection of claims 1-40 under 35 U.S.C. § 112, first paragraph, are therefore respectfully requested.

X. The Rejections Under 35 U.S.C. § 112, Second Paragraph, Are Traversed

In the Office Action at pages 10-11, the Examiner has rejected claims 1-40 under 35 U.S.C. § 112, second paragraph, for indefiniteness. Applicants respectfully traverse each of these rejections, in view of the foregoing amendments and the following remarks.

A. The Rejection of Claims 1-8 and 19-40

The Examiner contends that claims 1-8 and 19-40 are indefinite in the recitation of the term "capable of" since it is allegedly unclear what is encompassed by this phrase. Applicants respectfully disagree with these contentions. The present specification amply indicates that the vectors of the invention undergo replication in certain cells under certain conditions. *See, e.g.*, specification at pages 6-7, at page 14, and at pages 22-23. Specifically, by containing a gene essential for vector replication operably linked to a tissue-specific regulatory sequence the present vectors will undergo replication within a target cell only when the regulatory sequence has been activated or derepressed. Thus, the vectors of the invention are replication-*competent* (*i.e.*, they are "capable" of replicating), but they are also replication-*conditional* (*i.e.*, they are capable of replicating in certain cells only under conditions where the regulatory sequence is activated or derepressed). Therefore, one of ordinary skill would understand what is meant by a vector that is "capable of tissue-specific replication" -- *i.e.*, a vector that retains the ability to replicate in certain cells under certain conditions, or a "replication-conditional" vector that is "replication-competent" in certain cells under appropriate conditions. A vector that is "capable" of replication would thus be understood by one of ordinary skill to mean the converse of a "replication-deficient" or "replication -defective" vector (as those terms are also understood in the art) which

has been treated or has mutated such that it no longer can undergo replication under *any* conditions.

However, solely to expedite prosecution, Applicants have now amended claims 1, 19, 29, 30 and 40 (and thus the claims that depend therefrom) to delete the phrase "capable of tissue-specific replication" and to indicate that the vector of the invention is replication-conditional and undergoes tissue-specific replication. As indicated above, these amendments are fully supported in the specification as originally filed. Therefore, Applicants respectfully assert that this portion of the rejection has been overcome by the foregoing amendments and remarks. Reconsideration and withdrawal are therefore respectfully requested.

B. The Rejection of Claims 10 and 11

The Examiner also contends that claims 10 and 11 are indefinite for referring to the "vector" of claim 9, while claim 9 is a method claim. By the foregoing amendments, claims 10 and 11 have been amended to refer to the "method" of claim 9, thus fully accommodating this portion of the rejection. Reconsideration and withdrawal are therefore respectfully requested.

C. The Rejection of Claims 19-28 and 30-39

The Examiner also contends that claims 19-28 and 30-39 are indefinite in the recitation of "a cell" since it is not apparent whether the cell is an isolated cell or an implanted cell *in vivo*. Applicants respectfully traverse this rejection.

Independent claims 19 and 30, and thus the remaining claims which depend therefrom, are drawn to cells that have been transfected with a replication-conditional tissue-specific vector of the invention. That is, the claims are drawn to genetically engineered *cells*, regardless of whether

the cells are isolated or are implanted *in vivo*. Hence, Applicants respectfully assert that the recitation of "a cell" without any additional qualifier would allow one of ordinary skill to determine the metes and bounds of this claim, since it is the fact that the cell has been transformed and now contains a replication-conditional vector of the invention that is important, rather than whether the cell is isolated or is implanted *in vivo*.

However, solely to expedite prosecution of the present application, claims 19-28 and 30-39 have been amended by the foregoing amendments to now recite "an isolated cell," as suggested by the Examiner. As noted above, these amendments are fully supported in the specification and therefore do not add new matter. Accordingly, Applicants respectfully assert that this portion of the rejection has been overcome by the foregoing amendments. Reconsideration and withdrawal are therefore respectfully requested.

D. The Rejection of Claims 9-17

In rejecting claims 9-17, the Examiner contends that these claims are indefinite because

> . . . it is not apparent as to what is the stated effect of the distribution of a polynucleotide *in vivo* in accomplishing a beneficial effect. Furthermore, claim 17 is indefinite because it is not apparent as to what is the stated effect of *in vivo* gene expression of a heterologous gene in accomplishing a beneficial effect.

Office Action at page 10, last paragraph. Applicants respectfully disagree with these contentions.

The claims as amended are drawn to methods of distributing polynucleotides to cells and tissues. Contrary to the Examiner's contentions, there is no requirement that a claim recite a particular (or *any*) beneficial effect in order to meet the requirements of 35 U.S.C. § 112, second paragraph. Hence, this portion of the rejection is in error and should be withdrawn.

Moreover, even if such a requirement *did* exist under U.S. patent law (which it does not), the present specification as originally filed is replete with descriptions and definitions of the benefits of distributing a polynucleotide in cells and tissues *in vivo*, *ex vivo* and *in vitro*. For example, in the specification at page 12, lines 11-14, it is taught that :

> [t]he object of the distribution [of a vector and the gene(s) it contains] is to deliver the vector, gene product or the effects of the gene product . . . to substantially all or a significant number of cells of the target tissue, so as to treat substantially the entire target tissue.

It is also taught in detail in the specification that such treatment "is particularly necessary in cases in which surgical intervention is not feasible." Specification at page 21, lines 25-26. Furthermore, at pages 23-24 of the specification, it is taught that in certain embodiments of the invention the vector may encode a heterologous gene product, which may be toxic to the target cells; the distribution of such vectors in the cells in the targeted tissue leads to localized or long-range toxic effects within the tissue, such as prevention, inhibition, or destruction of the growth of the targeted cells or tissues. Finally, the specification teaches in detail that the distribution of polynucleotides in cells, including in cells *in vivo*, may be used to provide virus producer cells that facilitate the safe and efficient production of recombinant replication-conditional vectors which may be used, *inter alia*, for targeted gene therapy *in vivo*. *See* specification at pages 28-29.

Hence, Applicants respectfully assert that, contrary to the Examiner's contentions, there is no requirement for a claim to recite a beneficial effect in order to meet the definiteness requirements of 35 U.S.C. § 112, second paragraph. Moreover, even if such a requirement existed, one of ordinary skill would readily understand the beneficial effects of the distribution of a polynucleotide *in vivo*, and of *in vivo* gene expression of a heterologous gene, when viewing the language of claims 9-17 in the context of the specification as originally filed. Accordingly,

Claims 9-17 are not indefinite; reconsideration and withdrawal of this portion of the rejection are therefore respectfully requested.

E. The Rejection of Claim 18

The Examiner has also rejected claim 18, contending that this claim is indefinite because:

> it is not apparent as to what are the metes and bounds of the anti-tumor activity of "said heterologous gene product." Does the anti-tumor activity cure, prevent, stimulate, or inhibit the growth of a tumor in all target tissues *in vivo*?

Office Action at page 11, first paragraph. Applicants respectfully disagree with these contentions.

The metes and bounds of the anti-tumor activity of the heterologous gene product recited in claim 18 are clearly indicated in the specification as originally filed. For example, it is taught in the specification that "[t]he term 'anti-tumor activity' is intended to mean any activity which inhibits, prevents, or destroys the growth of a tumor." Specification at page 12, lines 6-7. Thus, one of ordinary skill reading claim 18 in view of the specification would readily understand that the anti-tumor activity of the heterologous gene product recited in claim 18 encompasses any activity having the effects on a tumor specified in the above-indicated definition of this phrase.

Accordingly, contrary to the Examiner's contention, claim 18 is not indefinite for reciting the phrase "anti-tumor activity." Reconsideration and withdrawal of this portion of the rejection are therefore respectfully requested.

F. Summary

In view of the foregoing amendments and remarks, Applicants respectfully assert that claims 1-40 particularly point out and distinctly claim the subject matter which Applicants regard as the invention. Reconsideration and withdrawal of the rejection of claims 1-40 under 35 U.S.C. § 112, second paragraph, are therefore respectfully requested.

XI. The Rejection Under 35 U.S.C. § 103 Over the Martuza '379 Patent Is Traversed

In the Office Action at pages 11-13, the Examiner has rejected claims 1-5, 8-14, 17-24, 27-35 and 38-40 as being unpatentable under 35 U.S.C. § 103(a) over U.S. Patent No. 5,728,379 to Martuza *et al.* (hereinafter "the '379 patent"). Applicants respectfully traverse this rejection.

As the Examiner states in the present Office Action, Tables 1 and 2 of the '379 patent indicate that a variety of tissue-specific promoters were known and available for use in vector constructs. Importantly, however, the information contained in Tables 1 and 2 of the '379 patent is *not* disclosed, suggested, or contemplated in U.S. Patent No. 5,585,096 ("the '096 patent") to Martuza *et al.* This information in the '379 patent is therefore new matter with reference to the '096 patent, and hence is only entitled to the filing date of U.S. Application No. 08/486,147 ("the '147 application") that ultimately issued as the '379 patent. The '147 application was filed on June 7, 1995, which is same date as the priority date of the present application by virtue of a claim of priority to U.S. Application No. 08/487,992. Therefore, the information contained in Tables 1 and 2 of the '379 patent, and all other matter in this patent that is not disclosed in the '096 patent, cannot be and is not prior art against the present application.

The '096 patent does not disclose, suggest, or contemplate the use of one or more tissue-specific promoters, such as α-fetoprotein, DF3, tyrosinase, CEA, surfactant, and ErbB2, in the construction of the claimed adenovirus vectors. The new information contained in the '379 patent that describes the use of a variety of tissue-specific promoters in constructing *Herpesvirus* vectors is not prior art to the present application. Hence, the disclosure of the '379 patent does not render obvious the claimed invention.

In view of the foregoing amendments and remarks, Applicants respectfully assert that a *prima facie* case of obviousness of claims 1-5, 8-14, 17-24, 27-35 and 38-40 over the '379 patent

has not been established. Reconsideration and withdrawal of this rejection are therefore respectfully requested.

XII. The Rejection Under 35 U.S.C. § 103 Over the Martuza '096 Patent Is Traversed

In the Office Action at pages 13-14, the Examiner has rejected claims 1-5, 8-14, 17-24, 27-35 and 38-40 as being unpatentable under 35 U.S.C. § 103(a) over the '096 patent. Applicants respectfully traverse this rejection, in view of the foregoing amendments and the following remarks.

In making this rejection, the Examiner contends that:

> Martuza *et al.* disclose and a method [sic] for killing tumor cells *in vivo* comprising administration of tissue-specific-replication competent herpes simplex virus vectors to tumor cells. The tissue-specific-replication competent herpes simplex virus vectors contain a tissue-specific or cell-specific transcriptional regulatory sequence that is operatively linked to an essential herpes simplex virus gene, wherein said transcription regulatory sequence effects expression of said gene in a specific tissue or cell, such that said virus replicates only in said tissue or cell. Columns 11 and 12 disclosed the tissue-specific-replication competent herpes simplex virus vectors and methods of using the herpes simplex virus vectors to express a heterologous gene for specific killing of tumor cells. Columns 15 and 16 provide a guidance as to how to construct and produce the tissue-specific-replication competent herpes simplex virus vectors. Examples 2-5 provide a detailed description as to how to use the replication-competent viral vectors in *in vivo* extracranial and *in vivo* intracranial tumor killing models. Given that tissue-specific promoters selected from group consisting of α-fetoprotein, DF3, tyrosinase, and ErbB2 are known in the art prior to the effective filing date of the as-file [sic] application (see Tables 1 and 2 of the '379 patent), it would have been obvious for one of ordinary skill in the art to have constructed and employed the tissue-specific-replication competent herpes simplex virus vectors of Martuza *et al.* using a known tissue-specific promoter operably linked to a viral gene necessary for herpes simplex virus replication for expressing a heterologous gene, *e.g.*, cytokines, in a tumor cell-specific fashion in order to target an immune response that kills the tumor cells.

> One of ordinary skill in the art would have a reasonable expectation of success in constructing and employing the tissue-specific-replication competent herpes simplex virus vectors of Martuza *et al.* for distributing and expressing a polynucleotide at a tissue *in vivo*, particularly given that columns 15, 16, and Examples 2-5 provide a detailed description as to how to construct and employ such vectors for killing tumor cells in a specific fashion.
>
> Thus, the claimed invention was within the ordinary skill in the art to make and use at the time it was made and was as a whole, *prima facie* obvious.

Office Action at page 13, line 7, to page 14, line 13. Applicants respectfully disagree with these contentions.

In proceedings before the Patent and Trademark Office, the examiner bears the burden of establishing a *prima facie* case of obviousness based upon the prior art. *See In re Piasecki*, 223 USPQ 785, 787-88 (Fed. Cir. 1984). The Examiner can satisfy this burden only by showing some objective teaching in the prior art or that knowledge generally available to one of ordinary skill in the art would lead that individual to combine the relevant teachings of the references. *See In re Fine*, 5 USPQ2d 1596,1598 (Fed. Cir. 1988). Furthermore, in order to support a *prima facie* case of obviousness, the prior art must suggest making the *specific* molecular modifications necessary to achieve the claimed invention. *See In re Deuel*, 51 F.3d 1552, 1558 (Fed. Cir. 1995). In the present case, this burden has not been satisfied.

The '096 patent is limited to disclosure of herpes simplex virus (HSV) vectors that may be used for killing malignant brain tumor cells. No other vector constructs, and most particularly no non-*Herpesvirus* vector constructs, are disclosed, suggested or contemplated in the '096 patent. Therefore, the '096 patent cannot and does not render obvious the presently claimed non-HSV vectors constructs, cells containing such constructs, and methods using such constructs.

In addition, the only heterologous (*i.e.*, non-viral) regulatory regions used in the constructs disclosed in the '096 patent are those regulating the expression of nestin, basic fibroblast growth factor (bFGF), and the epidermal growth factor receptor (EGFr). *See* '096 patent at column 11, lines 16-32. As one of ordinary skill would appreciate, nestin, bFGF and EGFr are not expressed in a "tissue-specific" manner as that term is defined in the present specification at pages 14-15. Moreover, there is no disclosure, suggestion, or contemplation in the '096 patent of the heterologous tissue-specific promoters used and claimed in the present invention, particularly those selected from the group consisting of α-fetoprotein, DF3, tyrosinase, CEA, surfactant, and ErbB2. Since it fails to disclose or suggest the presently claimed vectors or methods, the '096 patent is deficient as a primary reference upon which a *prima facie* case of obviousness of the present claims may be based. Claims 1-5, 8-14, 17-24, 27-35 and 38-40 therefore are not and cannot be rendered obvious by the '096 patent.

These deficiencies of the '096 patent are not cured by the additional disclosure found in the '379 patent. As noted above, the information in Tables 1 and 2 of the '379 patent is new matter with reference to the '096 patent. Nowhere in the '096 patent is there a disclosure that one or more tissue-specific promoters, such as α-fetoprotein, DF3, tyrosinase, CEA, surfactant, and ErbB2, could or should be used to make and use the adenovirus vectors of the present invention. Hence, the disclosures of the '096 and '379 patents do not disclose the claimed invention.

In fact, this statement can be carried farther -- beyond simply not *disclosing* the claimed vectors, cells and methods, there is absolutely no suggestion or contemplation in the '096 and '379 patents (beyond the above-noted material in Tables 1 and 2 of the '379 patent that is unavailable as prior art) that would have motivated one of ordinary skill in the art to insert one or more tissue-

specific transcriptional regulatory sequences described in the '379 patent into the HSV vectors of the '096 patent to obtain the presently claimed adenovirus vectors. Thus, the Examiner's contention that the combination of the '096 patent and Tables 1 and 2 from the '379 patent would have rendered the presently claimed vectors and methods obvious to the skilled artisan is pure speculation, since the Examiner has not pointed to any teaching or suggestion in the '096 patent, nor to any other permissible prior art reference or personal knowledge, as to what would have motivated the skilled artisan to do what Applicants have done. Absent such teaching, suggestion, and motivation, the '096 patent, alone or in combination with Tables 1 and 2 of the '379 patent, cannot support a *prima facie* case of obviousness.

In view of the foregoing amendments and remarks, Applicants respectfully assert that a *prima facie* case of obviousness of claims 1-5, 8-14, 17-24, 27-35 and 38-40 over the '096 patent has not been established. Reconsideration and withdrawal of the rejection under 35 U.S.C. § 103 over the '096 patent are therefore respectfully requested.

XIII. The Rejection Under 35 U.S.C. §§ 102(b) or 103 Over Babiss Is Traversed

In the Office Action at pages 14-15, the Examiner has rejected claims 1-8 and 19-42 under 35 U.S.C. § 102(b) as being anticipated by, or in the alternative under 35 U.S.C. § 103 as being obvious over, Babiss *et al.*, *J. Mol. Biol. 193*:643-650 (1987) (Doc. AR1, of record; hereinafter "Babiss"). Applicants respectfully traverse this rejection.

In making this rejection, the Examiner contends that:

> Babiss *et al.* teach a replication competent adenovirus where the promoter for albumin gene, a liver specific promoter, regulates the expression of E1a and E1b in liver cells (p. 645, col. 2, lines 1-19). Babiss *et al.* indicate that the vector can be used in assays to determine the effect of replication on the expression of endogenous genes (p. 649, col. 1, lines 4-8). Fig. 2 depicts

> transcription pattern in HepG2 cells after infection by alb194 [sic; abl194] virus. Column 2 at page 645 states that "injection with the abl454 virus, which includes the E1A enhancer upstream from the albumin promoter and results in a five fold increase in transcription rate from the exogenous albumin promoter on the virus". To the extent that the reference is ambiguous regarding the use of a tissue specific promoter selected from group consisting of α-fetoprotein, CEA, DF3, tyrosinase, and EbrB2, it would have been obvious for one of ordinary skill in the art at the time of the invention to make the adenoviral vector of Babiss *et al.* by employing any of the known tissue-specific promoters such as α-fetoprotein, CEA, DF3, tyrosinase, and ErbB2, particularly since Tables 1 and 2 of the '379 patent indicate that such promoters are known and available at the time the invention was made. Thus, absent evidence to the contrary, and in the alternative, the adenoviral vector or Babiss *et al.* has all of the properties cited in the claims.

Office Action at page 14, line 17, to page 15, line 12. Applicants respectfully disagree with these contentions.

In order to anticipate a claim, each and every element of the claim must be expressly or inherently disclosed in a single prior art reference. *See Kalman v. Kimberly Clark Corp.*, 713 F.2d 760, 771 (Fed. Cir. 1983), *cert. denied*, 465 U.S. 1026 (1984). Thus, in order to anticipate claims 1-8 and 19-42 under 35 U.S.C. § 102(b), Babiss must expressly or inherently disclose tissue-specific replication-conditional adenovirus vectors comprising a heterologous tissue-specific transcriptional regulatory sequence operably linked to the coding region of a gene that is essential for replication of the vector (claims 1-8), and an isolated cell containing such a vector wherein the transcriptional regulatory sequence functions in the cell so that replication of the vector occurs in the cell (claims 19-42). Key to the analysis of whether or not Babiss is an anticipatory reference is the requirement for a *replication-conditional* vector in the present claims, an element that is not present in the vectors described in Babiss.

As fully described in the specification at page 14, lines 1-17, a "replication-conditional vector" is a vector that will not replicate in a cell or tissue except when the transcriptional

regulatory sequence in that vector is activated or derepressed. That is, the vectors of the invention are capable of replication under certain conditions within a cell or tissue, *i.e.*, when transcriptional regulatory activators are present, or transcriptional regulatory inhibitors absent, within the cell or tissue in which the vector resides. Importantly, however, the vectors of the present invention are fully capable of replication without the aid of a helper virus -- under the above-noted conditions of activation or derepression, the vectors will replicate regardless of the presence of a complementing sequence contributed by the cell, tissue, or another virus. Hence, the vectors of the invention may be considered to be replication-*competent*. In at least this respect, the vectors of the invention therefore are quite different from those disclosed in Babiss, which are replication-*incompetent*. *See, e.g.*, Babiss in the Abstract at page 643, lines 1-2 (indicating that "non-replicating adenovirus genomes" were used); in the Introduction at page 644, col. 1, lines 11-13 (indicating that "recombinant adenoviruses containing cell promoters that can replicate with the aid of a helper virus" were used); and in the Results at page 645-646 and in Figures 2 and 3 (indicating that the alb194 and alb454 viruses did not replicate in transfected cells unless the cells had been co-transfected with the dl313 helper virus). Clearly, then, Babiss discloses only replication-incompetent vectors, and not the replication-competent vectors required by the present claims.

Thus, one of ordinary skill, viewing the Babiss reference as a whole, would appreciate that the alb194 and alb454 viruses of Babiss, cited by the Examiner in this portion of the rejection as noted above, are replication-*incompetent* and therefore could not be used to make and use the presently claimed vectors, cells and methods which require replication-*competent* vectors. Hence, Babiss cannot and does not anticipate claims 1-8 and 19-42 under 35 U.S.C. § 102(b).

The Examiner also attempts to combine the disclosure of the '379 patent with that of Babiss to support an alternative rejection of claims 1-8 and 19-42 under 35 U.S.C. § 103. In this regard, Applicants reiterate and incorporate by reference herein the remarks made above regarding the '379 patent. The information contained in Tables 1 and 2 of the '379 patent is not available as prior art against the present application. Hence, the alternative rejection of claims 1-8 and 19-42 under 35 U.S.C. § 103 over Babiss in combination with the '379 patent is improper and must be withdrawn.

In view of the foregoing amendments and remarks, Applicants respectfully assert that claims 1-8 and 19-42 are not unpatentable under 35 U.S.C. § 102(b) or in the alternative under 35 U.S.C. § 103 over Babiss. Reconsideration and withdrawal of this rejection are therefore respectfully requested.

XIV. The Provisional Rejection Under the Judicially Created Doctrine of Obviousness-Type Double Patenting Is Traversed

In the Office Action at pages 15-16, the Examiner has provisionally rejected claims 1-40 under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-42 of commonly owned U.S. Application No. 08/487,992 ("the '992 application"). In making this rejection, the Examiner states, at page 16, lines 5-10:

> [a]lthough the conflicting claims are not identical, they are not patentably distinct from each other because both set of claims are directed to drawn to [sic] a tissue-specific replication-conditional vector, isolated cells containing the tissue-specific replication-conditional vector, method of producing the vectors, and methods of using the tissue-specific replication-conditional vector for distributing a polynucleotide in a tissue *in vivo*.

As Applicants informed the Examiner during the interview on August 12, 1998, the '992 application will be abandoned in favor of the present application, thereby obviating any double-

patenting issues that may exist between claims 1-42 of the '992 application and claims 1-40 of the present application. Reconsideration and withdrawal of this rejection are therefore respectfully requested.

XV. Summary

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding rejections and that they be withdrawn.

Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this amendment and reply are respectfully requested.

Respectfully submitted,

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Date: October 13, 1998

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